Reference: *Biol. Bull.*, **146**: 206–216. (April, 1974)

THE BIOLOGY OF *ASCIDIA NIGRA* (SAVIGNY) IV. SEASONAL AND SPATIAL PATTERNS OF EMBRYONIC DEVELOPMENT AND HATCHING SUCCESS

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In previous papers one of us has drawn attention to the fact that in Jamaica *Ascidia nigra* breeds throughout the year but apparently has peaks of reproductive activity at certain times, particularly in the winter months (Goodbody, 1961, 1965). The data on which these conclusions are based were drawn from the appearance of young ascidians on test plates examined at weekly intervals. There is no accurate method of studying spawning behavior in the wild and, as the developmental time from fertilization to settlement of the larva can be as short as twelve hours, it is not possible to monitor reproductive cycles by following changes in the population of larvae in the plankton. In any case the eggs and larvae are virtually indistinguishable from certain other related species. There remains therefore an uncertainity as to whether the changing numbers of young ascidians appearing on plates is due to actual differences in the numbers of eggs released or is due to differing patterns of survival in eggs, embryos and larvae during the planktonic phase of their existence. In order to examine this possibility we have followed, on an experimental basis, the seasonal changes in embryonic survivorship when the eggs are allowed to develop in three different types of water: open ocean water, harbor water and mangrove-lagoon water.

By rearing the eggs in these different types of water we have been able to throw light on one further aspect of the biology of this species. *Ascidia nigra* is most commonly found in inshore regions both in the mangrove lagoons and in Kingston Harbour, but it is exceedingly rare to find it on coral reefs. The absence of this species from coral reefs could be due to the inability of embryos and larvae to survive in the type of water found in the open ocean and neighborhood of the reefs, or it could be due to the inability of the adult zooid to survive and grow in this environment. The experiments outlined in this paper will show that far from being unable to survive in this type of water the embryos survive better here than in water from inshore areas.

For technical reasons it was not possible in these experiments to study the survival of larvae after hatching and our data relate only to the period from fertilization to hatching. However, there is no reason to suppose that larvae will not survive in conditions in which the embryos have successfully developed, and we consider that the picture of survivorship given in this paper will apply to the whole period of early development up to the moment when the larva finally selects a site for attachment. It is also important to remember, when considering these data, that they are derived entirely from laboratory experiments under conditions free from competition or predation. The actual pattern of survivorship occuring in the wild could be quite different if there are any marked seasonal differences in predation pressure on the embryos and larvae. At present we do not know anything about the type of predation involved and nor do we know sufficient about seasonal changes in the planktonic carnivores to be able to consider this aspect of the problem.

Materials and Methods

The water used in these experiments came from three different places in the vicinity of Port Royal, Jamaica. Open Ocean water was collected from adjacent to East Middle Ground Shoal (Long. 76° 47.1′ Lat. 17° 54.6′); Port Royal water was collected from Port Royal Harbour (Long. 76° 50.6′ Lat. 17° 56.4′); Mangrove Lagoon water was collected from Fort Rocky Lagoon (Long. 76° 49.5′ Lat. 17° 56.3′). Locations can be found on a map in Goodbody (1970) or on British Admirality Chart No. 454. Water was collected freshly each week in glass containers kept specially for this purpose. Following collection each water sample was filtered through a membrane filter (0.4 μ) and stored for use in glass containers with glass stoppers at a temperature of 28° C. The water was always used for experiments on the day following collection and filtration.

Artificial sea water was used for collecting eggs and sperm prior to fertilization. Instant Ocean (Ocean Systems Inc.) was used for this purpose. A large container was always available for use and samples for each experiment were filtered in the same way and at the same time as the other samples. As the objective of the experiments was to compare the success of development of eggs from a single animal in three different types of natural sea water it was necessary to find a "neutral" water in which to collect the eggs and sperm.

In the genus *Ascidia* eggs are shed from the ovary into a long oviduct which follows the curvature of the post-intestine and opens just posterior to the anus. When full of eggs this appears as a creamy white duct on the surface of the animal. The sperm duct accompanies the length of the oviduct, is deeper down in the tissues and is snowy white in color. After the oviduct is drained of eggs the sperm duct is clearly visible beneath. Animals used for fertilizations were first removed from the test and the heart was pierced and drained of blood, thus helping to prevent contamination of the water by blood. The animal was dried on filter paper and the oviduct pierced about half way along its length and eggs gently squeezed into a dish of water. The sperm duct was then pierced with a glass needle and the sperm drained into a separate dish of water. Fertilization was always between different animals.

Eggs and sperm collected in this way remain held in strings so that in each case it is possible to draw them back into a pipette with minimum contamination by the artificial sea water. Glass pipettes were thus used to transfer eggs from each animal into three separate dishes containing respectively Open Ocean water (OO), Harbor water (PR) and Mangrove Lagoon water (ML). Similarly the sperm was also transferred to three separate dishes. Fertilization was effected by pouring an appropriate dish of sperm (*i.e.*, from the same water type) into a dish of eggs and pouring back and forth between the dishes until the string of eggs was completely dispersed and mixed with sperm. Sperm and eggs were allowed to remain together for thirty minutes before the excess sperm was decanted off and the dish refilled with the appropriate water. All dishes were etched to indicate a level equalling 75 ml of water in the dish, so that all batches of eggs developed in a similar volume

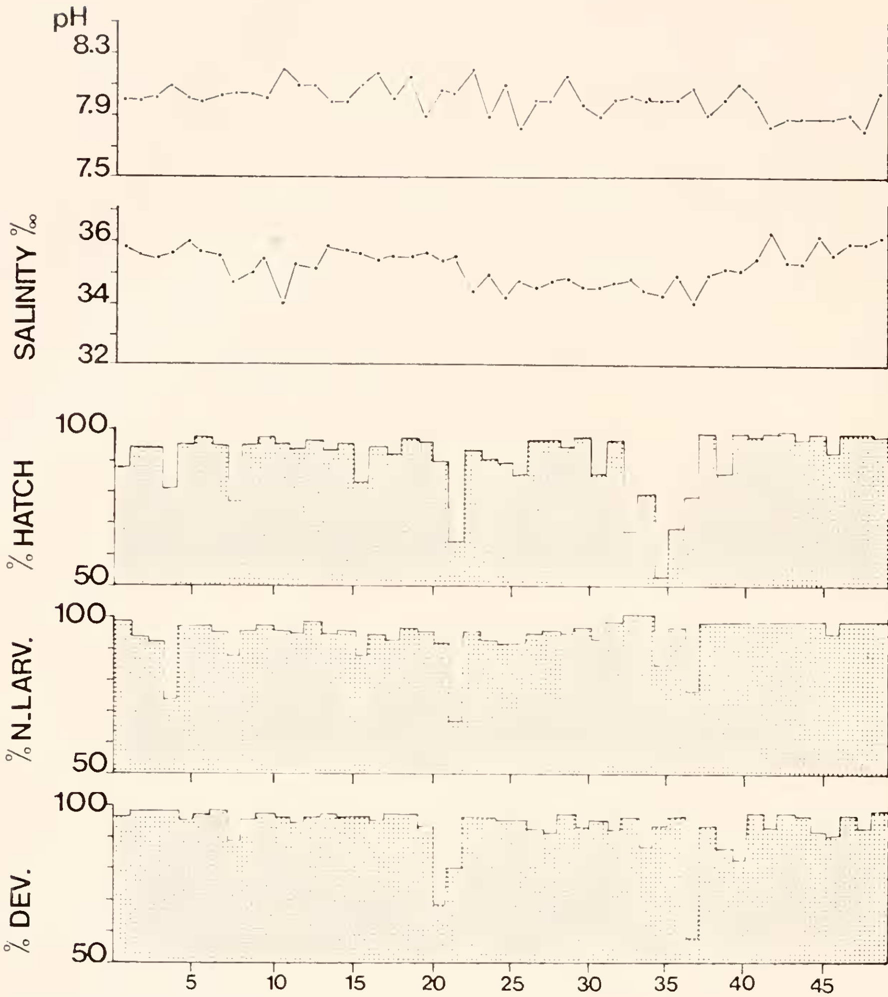

FIGURE 1. Percentage success in the development of eggs (Dev.), development of normal larvae (N-Larv.) and hatching (Hatch) in *Ascidia nigra* over a period of one year commencing April 1969 when reared in Open Ocean water. Horizontal scale shows time in weeks (week 10 = June, week 20 = September, week 30 = November, week 40 = February). Upper figures show pH and salinity of the water. All embryos were developd at 28° C (For further detail see text).

of water. Before being placed in the incubator each dish was inspected visually and the quantity of eggs reduced with a pipette until approximately 500 eggs remained, thus ensuring a similar number of eggs in each batch. Because the eggs are floating freely at this stage in a comparatively large volume of water this procedure could never be more than approximate. However, test experiments have shown that over two thousand embryos can develop successfully together in 75 ml of water without any lowering of survival.

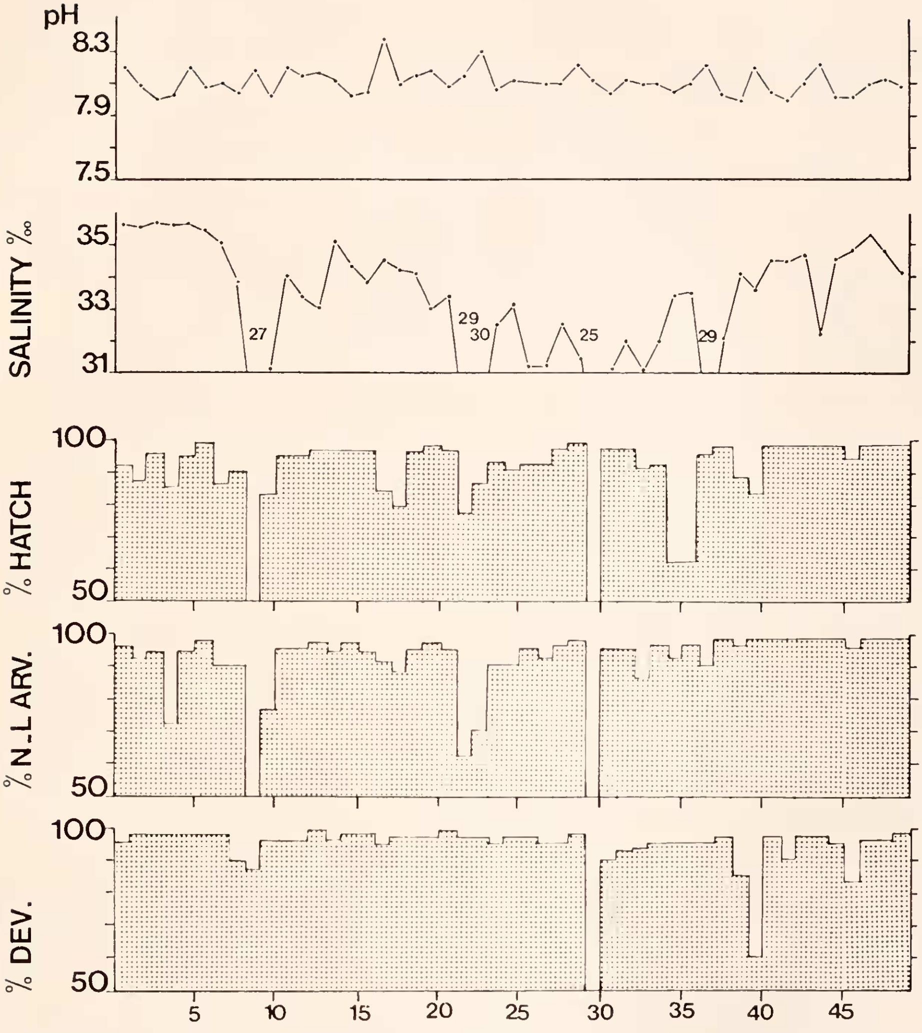

FIGURE 2. Percentage success in the development of eggs (Dev.), development of normal larvae (N-Larv.) and hatching (Hatch) in *Ascidia nigra* over a period of one year commencing April 1969 when reared in Inshore Harbor water. Horizontal scale shows time in weeks (week 10 = June, week 20 = September, week 30 = November, week 40 = February). Upper figures show pH and salinity of the water. All embryos were developed at 28° C (For further detail see text).

As soon as the number of eggs and the volume of water had been adjusted the dishes were covered with glass plates and placed in an incubator at 28° C (mean sea temperature at Port Royal). Ten hours after fertilization the contents of every dish were transferred to separate containers and fixed by the addition of neutral formalin. Each sample was later examined with an inverted microscope and total counts made of the following: (a) undeveloped eggs (b) developed eggs (c) normal

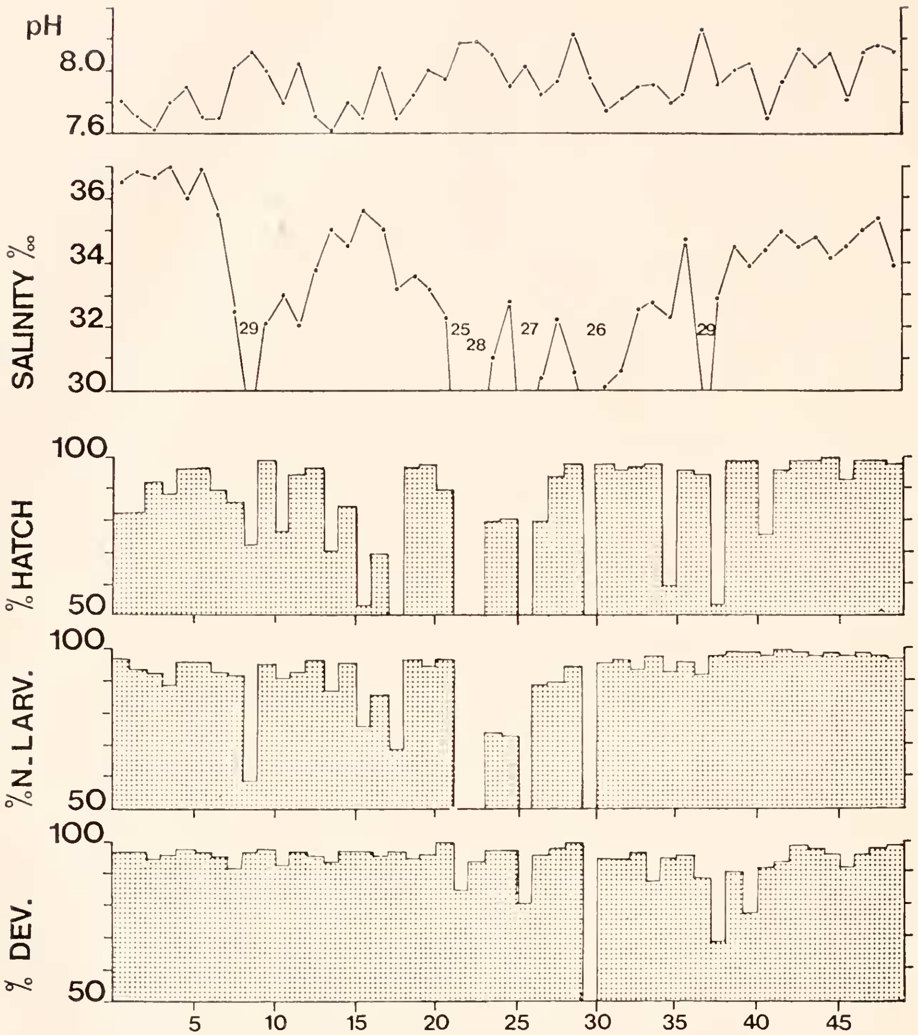

FIGURE 3. Percentage success in the development of eggs (Dev.), development of normal larvae (N-Larv.) and hatching (Hatch) in *Ascidia nigra* over a period of one year commencing April 1969 when reared in Mangrove Lagoon water. Horizontal scale shows time in weeks (week 10 = June, week 20 = September, week 30 = November, week 40 = February). Upper figures show pH and salinity of the water. All embryos were developed at 28° C (For further detail see text).

larvae (d) abnormal larvae (e) unhatched larvae. The data presented in this paper, and based on these figures, show only (a) the percentage of eggs which showed any development at all, (b) the number of normal larvae expressed as a percentage of the total number of eggs which developed, (c) the percentage of all larvae which successfully hatched.

All glass-ware except pipettes, used during the fertilization and development procedures was washed with Haemosol, rinsed in tap water and then in glass distilled water. Pipettes were cleaned with chromic acid and then rinsed as above.

Each weekly experiment consisted of six separate fertilizations treated as described above and using fresh animals for each new fertilization.

Results

Data for forty-nine experiments carried out at approximately weekly intervals throughout a year are presented in Figures 1, 2 and 3. In each case histograms are shown to illustrate the percentage of eggs which showed any development at all: the percentage of developing eggs which produced normal larvae, whether or not they actually hatched: and the percentage of developing eggs which actually hatched. Data are also presented to show the salinity and pH of the water used on each occasion. These data may be interpreted under three separate headings.

Development

It is clear that mature eggs are available throughout the year and that some degree of development is always possible. This is most clearly illustrated in Figure 1 for eggs developing in open ocean water. If we assume that there might always be about 10% failures and consider only those occasions when less than 90% of eggs show any development, we find that serious failures of development in open ocean water only occurred in weeks 21, 22, 37 and possibly 39 and 40. In weeks 21, 22 and 37 more than 90% eggs developed in Port Royal water. In week 39, 90% eggs developed in mangrove water. In week 40 alone was there any general effect, when only 65% of all eggs developed irrespective of the water type in which they were reared. Nevertheless it is reasonable to conclude that mature eggs are always available and capable of development.

Normal larvae

Frequently ascidian larvae will develop with imperfect or distorted tails and it is these which are here considered as abnormal. It is clear that normal larvae can be produced throughout the year. In open ocean water large numbers of abnormal larvae (*i.e.* less than 90% normal larvae) occurred in weeks 4, 22, 33, 35 and 37. In weeks 4, 33, 35 and 37, 90%, or almost 90%, normal larvae were produced when developed in mangrove water. In week 22 there was a general effect and only 42% of all larvae were normal irrespective of the water type used. This is undoubtedly an environmental effect and not a physiological incapacity in the animal. The week in question was at the beginning of the rainy season and very heavy rain had occurred in the previous week, probably flushing considerable quantities of material off the land.

Hatching success

The greatest variability is to be found in hatching success, particularly in the mangrove water. Nevertheless if hatching is examined through all of the water types we find that a high degree of success is possible throughout the year. For comparative purposes we have taken 80% success as a base line and consider only major departures from this. In open ocean water such departures are to be found in weeks 8, 22, 33, 35, 36 and 37. In week 8, 85% hatched in mangrove water, in weeks 33, 36 and 37, 96% hatched in mangrove water. In week 22, almost 80% hatched in harbor water. In week 35 alone was there a fairly general departure from the base line when only 58% of all developed eggs successfully hatched irrespective of water type.

The above analysis of the data relates embryonic survival to the season of the year, but the data may also be examined so as to compare success in the three different types of water. When this is done it becomes obvious that the highest degree of success is achieved by rearing eggs in open ocean water and the least success is obtained with mangrove water (see Table I). Similarly it will be noted from Figures 1–3 that in terms of salinity and pH, the open ocean is the most stable environment while the mangrove lagoon is the least stable. In order to examine the causes of variability all instances in which hatching success is less than 75% have been examined in relation to salinity and pH of the water in question. The data are presented in Table II.

TABLE I

Overall success of development, production of normal larvae and hatching in three types of water Data are expressed as the mean percentage of all experiments carried out in the water type in question (See text).

Water type	Development	Normal larvae	Hatching
Open Ocean	92.7%	93.9%	90.3%
Harbor	92.8%	89.0%	88.0%
Mangrove	91.4%	84.4%	80.5%

The data in Figures 1–3 indicate that a high degree of survival is possible at salinities down to about 29.0 ppt but that below that survival is invariably poor. Inspection of Table II shows that in the open ocean salinities never dropped to this level, in the harbor on only two occasions out of four, and in the mangrove lagoon on four occasions out of ten. Salinity changes therefore, cannot be the only factor responsible for poor hatching success, although when salinity does fall below 29 ppt there is almost complete failure of development and hatching.

Berrill (1929) has shown that hatching in the Ascidiidae is inhibited at low pH values. In his experiments with *Phallusia mammilata* he found total inhibition below pH 6.8 but 95% of embryos hatched successfully at pH 7.2. Although values as low as this never occurred in our experiments it still remains possible that lowered pH may have been responsible for poor hatching in some instances in mangrove water (Table II). However, this cannot be the case for the instances of poor hatching in open ocean water and harbor water. Of these there was poor hatching in both water types in weeks 35 and 36 and it seems likely that there was some common factor at work. No plausible reason can be put forward for poor hatching in open ocean in week 33, but week 22 coincided with the beginning of the September rains and, as already mentioned, there may at this time have been considerable run off from the land of toxic substances such as insecticides accumulated during the dry season.

The data presented so far make it clear that *Ascidia nigra* is capable of producing viable eggs at all seasons of the year and that these eggs are able to develop successfully. However, the physiological ability of the animal to produce viable eggs does not imply that it will spawn. In an attempt to throw some light on the question of spawning cycles we kept records of the state of the gonoducts in weekly samples of twenty animals over a period of two years. An arbitary scale was used from 0 to 5 to indicate the condition of the oviduct from completely empty (0) to very distended (5). Figure 4 shows for the year 1968/69 the percentage of oviducts in each sample which were full of eggs, *i.e.*, stages 4 and 5. The animals in question were all collected in the vicinity of Port Royal Harbour and it is clear from the figure that within this population there appear to be regular cycles of activity in which the ducts fill and empty. If we assume that the sample used each week is representative of the whole population in the area, and this may be an unwarranted assumption, then it would seem that spawning is in fact continuing throughout the year. The data for the year 1969/70 showed similar but less dramatic cycles of activity.

Table II

Salinity and pH in relation to lowered hatching success. For explanations see text.

Water type	Week no.	Percentage hatching success	Salinity	pH
Open ocean	22	64	35.0	8.05
	33	67	34.75	8.03
	35	52	34.2	8.0
	36	68	34.9	8.0
Harbor	9	0.03	27.7	8.18
	30	0.00	25.6	8.12
	35	62	33.4	8.05
	36	63	33.5	8.10
Mangrove	9	72	29.5	8.12
	14	70	35	7.62
	16	53	35.6	7.7
	18	34.8	33.2	7.7
	22	0.00	25.7	8.17
	23	34.5	28.6	8.18
	26	3.5	27.6	8.02
	30	0.0	26.3	8.00
	35	58	32.3	7.8
	38	53	32.9	7.68

Discussion

The data presented in this paper can leave no doubt about the fact that mature eggs of *Ascidia nigra* are available throughout the year in Jamaica and that there

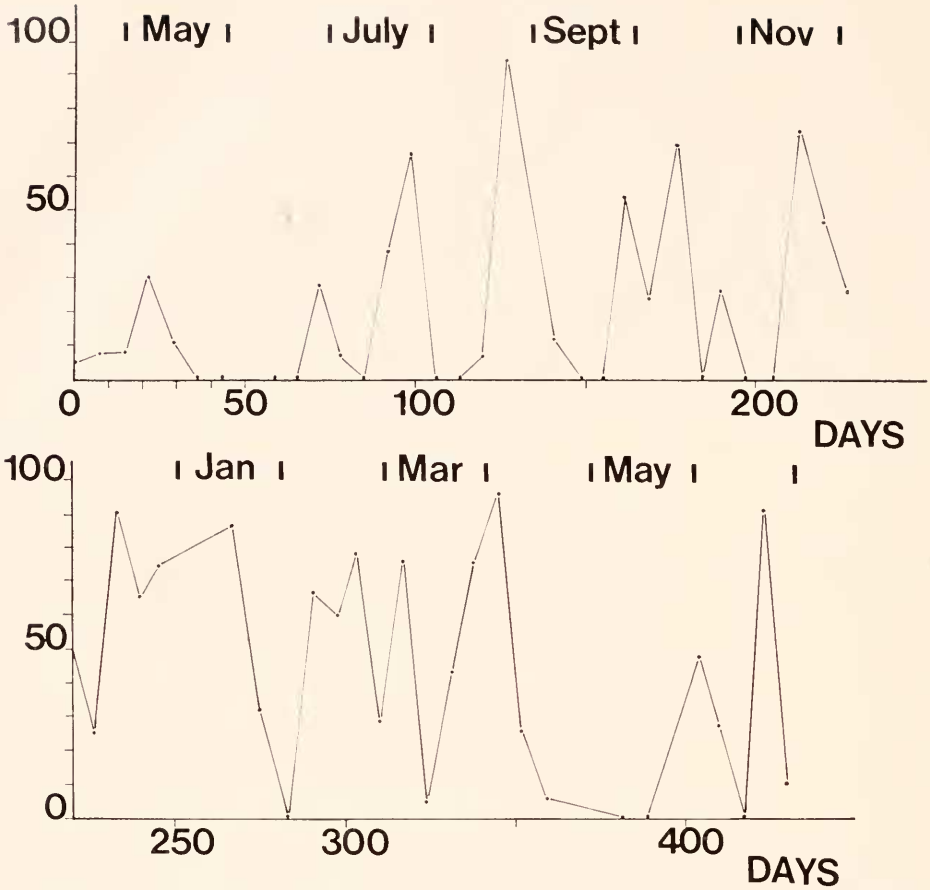

FIGURE 4. Percentage of gonoducts in stages 4 and 5 (*i.e.* full and distended with genital products) in weekly samples of *Ascidia nigra* from April 1968 to June 1969.

is no season of the year when, for physiological reasons, such eggs are unable to develop. This is most clearly demonstrated when eggs are developed in open ocean water, in which there are very few occasions when survival was poor. When survival was poor in open ocean water survival was usually high in one of the other water types.

Nevertheless the fact that viable eggs are always available and can always develop successfully does not in itself permit us to conclude that the fluctuations in numbers of young ascidians appearing on test panels, as illustrated by Goodbody (1961, 1965), is not due to seasonal differences in reproduction but must be due to other causes. It is still possible that, while mature eggs are always available, animals do not actually spawn at all seasons. The cyclical nature of activity in the gonoducts, which is also confirmed from older unpublished data, is difficult to interpret. It might indicate that spawning is wholly cyclical: that eggs are produced continuously to fill the duct and that this is followed by total emptying of the duct in a single process. Alternatively, it may be that when the duct is full there is a continuous discharge of gametes at a relatively slow rate, keeping pace with egg production. In the latter case the presence of empty ducts would indicate the aftermath of a sudden burst of increased activity. A great deal more work needs to be done to elucidate the precise nature and timing of spawning cycles in this species.

On the basis of the data now available to us we may conclude that in this species in Jamaica spawning occurs throughout the year on a cyclical basis and that this may in part account for the seasonal differences in settlement of larvae already referred to. There are no seasonal differences in patterns of survivorship in embryos which might in any way account for the observed patterns of larval settlement. In another paper (Goodbody and Gibson, 1974) it will be shown that differences in the patterns of survival in post-metamorphic juvenile ascidians are, however, an important contributing factor in accounting for the observations recorded by Goodbody (1961, 1965).

We pointed out in the introduction to this paper that *Ascidia nigra* is very abundant in inshore harbor waters but is rare in open ocean environments such as coral reefs. The absence of the species in these environments could be due to a failure of the embryos to develop or a failure of the post-metamorphic ascidian to survive. It is clear from the data presented in this paper that there can be no inhibition of embryonic development in open ocean water and that its absence from coral reefs must be due to other factors.

The distribution of ascidians in various habitats in Jamaica including coral reefs will be discussed by one of us in a later paper, but a few general points are pertinent in relation to the survival of *Ascidia nigra*. On the reef crest and in the lagoon (*cf.* Goreau 1959 for terminology) only those species of ascidian occur which can survive underneath stones and most of these are encrusting colonial forms. On reef butresses and on the fore reef slope ascidians are rare and confined to a few species including *A. sydneiensis* and *Pyura vittata*. Many more species and individuals are to be found in the deep reef beyond 40 meters. In inshore environments *Ascidia nigra* is always found raised off the substratum on rocks, piers, boat moorings *etc.*, but never embedded under stones or within the sediments. Within the reef system one would therefore not expect to find it in shallow water but only on the fore-reef slope. Its absence from the reef may be due to competition from other organisms and in particular corals which may prey directly on the larvae before they settle. Similarly newly settled larvae will be subject to intense predation pressure by reef dwelling fish (Bakus, 1964). However there is other evidence which shows that *Ascidia nigra* may be unable to succeed in the open ocean for other reasons. Piers, boat moorings and marker buoys within the harbor system are usually heavily encrusted with *A. nigra* but such structures placed outside of the harbor seldom have this species attached to them. *A. nigra* is probably very dependent on phytoplankton for its food and requires large quantities such as are only to be found in very productive waters, as in the harbor and lagoons.

This work was supported by Grant No. GB 7079 from the U. S. National Science Foundation. The final manuscript was completed while the senior author was on sabbatical leave at the Plymouth Laboratory of the Marine Biological Association of the U. K. Acknowledgment is made to the Director of that laboratory for providing facilities and to the Nuffield Foundation for financial support.

Summary

1. The development of embryos of *Ascidia nigra* has been studied over a period of one year. Embryos were reared in water taken from three distinct areas, the open ocean, inshore harbor and mangrove lagoon.

2. Eggs are available throughout the year and are always capable of successful development. Spawning probably occurs on a cyclical basis at least once per month.

3. Embryos develop and hatch more successfully in open ocean water than in inshore waters. Differences in success of development and hatching are in part accounted for by differences in salinity and pH, but other factors may also be involved.

4. There are no distinctive seasonal differences in embryonic survival.

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